

tsRFinder

Requirements:

- Linux system, enough disk space and Ram depending on the size of RNA deep sequencing data. (Tested system: ubuntu 12.04 LTS, ubuntu 16.04 LTS)

Installation

- Download tsRFinder pipeline package from <https://github.com/zhlingl/tsRFun>

```
wget https://github.com/zhlingl/tsRFun/archive/refs/heads/main.zip
```

- Download necessary software, packages and reference databases as listed below:
 - Perl 5 (<https://www.perl.org>) (Tested version: v5.14.2, v5.22.1); Perl 5 might be already installed in the linux system.
 - Bowtie [1] (<http://bowtie-bio.sourceforge.net/index.shtml>) (Tested version: 1.1.1, 1.2.1.1)
 - Bedtools(<https://bedtools.readthedocs.io/en/latest/>) (Tested version: 2.25.0, 2.29.2)
 - Samtools(<http://www.htslib.org/>) (Tested version: 1.9, 1.13)
 - Reference database (See lists and download link of all pre-compiled species' databases in Pre-compiled Databases Instruction)
- Download tsRFinder pipeline package

- Install tsRFinder from <https://github.com/zhlingl/tsRFun>

- Unpack tsRFinder package

```
unzip tsRFun-master.zip
```

- Attach the tsRFinder directory to your PATH:

```
echo 'export PATH=$PATH:your_path_to_tsRFun-master/tsRFinder' >> ~/.bashrc.  
chmod 755 your_path_to_tsRFun-master/tsRFinder/tsRFinder.pl
```

- Install Bowtie

- Unpack bowtie-1.x.x-linux-x86_64.zip.

```
unzip bowtie-1.x.x-linux-x86_64.zip
```

- Attach the bowtie directory to your PATH:

```
echo 'export PATH=$PATH:your_path_to_bowtie' >> ~/.bashrc  
your_path_to_tsRFun-master/source/ tsRFun.pl
```

chmod 755

- Start a new shell session to apply changes to environment variables:

```
source ~/.bashrc
```

- Install bedtools

- Unpack bedtools-2.25.0.tar.gz.

```
wget https://github.com/arq5x/bedtools2/archive/v2.25.0.tar.gz
tar xzvf v2.25.0
cd bedtools2-2.25.0/
make
cd bin/
```

- Attach the bedtools directory to your PATH:

```
echo 'export PATH=$PATH:your_path_to_bedtools' >> ~/.bashrc
```

- Start a new shell session to apply changes to environment variables:

```
source ~/.bashrc
```

- Install samtools

- Unpack bedtools-2.25.0.tar.gz.

```
wget -c https://github.com/samtools/samtools/releases/download/1.9/samtools-1.9.tar.bz2
tar jxvf samtools-1.9.tar.bz2
cd samtools-1.9/
./configure --prefix= your_path_of_samtools (example:
/home/vip47/biosoft/samtools-1.9)
make
make install
```

- Attach the samtools directory to your PATH:

```
echo 'export PATH=$PATH:your_path_to_ samtools' >> ~/.bashrc
```

- Start a new shell session to apply changes to environment variables:

```
source ~/.bashrc
```

- Install perl modules

```
cpan -i Net::Server;
cpan -i Getopt::Std;
cpan -i File::Find;
cpan -i File::Basename;
cpan -i Cwd;
cpan -i Math::CDF;
cpan -i FileHandle;
```

- Download tsRFinder pipeline package

- Test if everything is installed properly:

```
perl -v
tsRFinder.pl -h
bowtie
samtools
bedtools
```

- If you get any error messages you should install the software or perl modules once again.

Script description

- The input files of tsRFinder.pl are:

Options:

```
-i <file>      Input could be: .fastq/.fq or .fasta/.fa file.  
-o<file>      Output address of annotation results  
-t <int>      Number of threads to launch (default = 4)  
-x <str>      Address of bowtie index tRNA information
```

Alignment:

```
-l <int>      The minimal length of the output sequences (default = 15)  
-L <int>      The maximal length of the output sequences (default = 45)  
-M <int>      The total number of mismatches in the entire alignment (default = 0)  
-p <float>    The p-value threshold to determine whether the fragment is tsRNA.
```

Others:

```
-v            Print version information  
-h            Print this usage message
```

Example of use:

```
tsRFinder.pl -i PATH_of_example/fasta -o PATH_of_example/ -x hg38_index/
```